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α-Lactalbumin and the Lactose Synthetase Reaction*

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SUMMARY

An improved procedure for the isolation of the A protein of lactose synthetase is presented. The K_m for UDP-galactose is not influenced by the concentration of glucose or α-lactalbumin but these compounds alter the maximum velocity of the reaction. There is a reciprocal relationship between the concentration of glucose and α-lactalbumin in the lactose synthetase assay and α-lactalbumin lowers the apparent K_m of glucose. It is suggested that the physiological function of α-lactalbumin is to lower the K_m of glucose so that it may be used maximally for the synthesis of lactose. Lactose may be synthesized at maximum rates by the A protein in the absence of α-lactalbumin but in the presence of high concentrations of glucose. Different preparations of the A protein have variable activities.

Lactose synthetase (UDP-galactose:D-glucose 1-galactosyl transferase, EC 2.4.1.22) catalyzes the biosynthesis of lactose (Equation 1):

$$\text{UDP-galactose} + \text{glucose} \rightarrow \text{lactose} + \text{UDP} \qquad (1)$$

The enzyme was first described by Watkins and Hassid (1) as a particulate enzyme in rat and guinea pig mammary glands. A similar enzyme was found as a soluble enzyme in bovine milk (2) and was partially purified by Babad and Hassid (3). Glucose was the principal galactosyl acceptor although *N*-acetylglucosamine was 25% as effective as glucose. Brodbeck and Ebner (4) resolved the soluble bovine lactose synthetase from milk into two protein fractions, designated as A and B, which were required for catalytic activity. The B protein was identified as α-lactalbumin, the common milk whey protein (5, 6). The B protein (α-lactalbumin) was found both in the microsomal and soluble portion of the cell whereas the A protein was found mainly in the "microsomal" fraction (7). Recently, Coffey and Reithel (8, 9) have shown that the A protein is in the Golgi apparatus and that the particles are similar in size to lysosomes and secretory granules. Palmiter (10) has described the properties of lactose synthetase from mouse mammary gland and has proposed the involvement of a third component in crude preparations.

Brew, Vanaman, and Hill (11) have shown that the A protein catalyzes the following reaction (Equation 2):

$$\text{UDP-galactose} + N\text{-acetylglucosamine} \rightarrow N\text{-acetyllactosamine} + \text{UDP} \qquad (2)$$

This reaction is inhibited by α-lactalbumin and the proposal was made that the B protein (α-lactalbumin) changes the specificity of the A protein so that Reaction 1 is catalyzed. An enzyme system which makes low amounts of lactose was found in other tissues (12) and, indeed, Ziderman *et al.* (13) have shown that small amounts of lactose are formed by particulate fractions isolated from rabbit gastric mucosa. Furthermore, the synthesis of lactose in such particles is greatly accelerated by the addition of bovine α-lactalbumin (see text). This paper describes a detailed purification procedure of the A protein and the relationship of α-lactalbumin in the lactose synthetase reaction.

EXPERIMENTAL PROCEDURE

Materials—Skim milk was obtained from the University Dairy. α-Lactalbumin (B protein) was purified as previously described (6). Calcium phosphate gel was prepared by the procedure described by Keilin and Hartree (14) and hydroxylapatite (aged at least 1 month) by the procedure of Siegelman, Wieczorek, and Turner (15). Cellulose phosphate was from Mann and DEAE-cellulose (DE-32) was from Whatman. *N*-Acetylglucosamine, NADH, phosphoenolpyruvate, glucose, glycylglycine, UTP, ATP, 2-mercaptoethanol, and pyruvate kinase (type I) were from Sigma. *N*-2-Hydroxyethylpiperazine-*N*-2-ethanesulfonic acid was from Calbiochem. UDP-galactose was synthesized by the method of Roseman *et al.* (16). Antiserum to bovine α-lactalbumin was purchased from Antibodies, Inc., Davis, California. Other chemicals were of reagent quality.

Definitions of Enzymatic Activities—The following definitions are used to describe the various reactions of the A protein in the absence or presence of α-lactalbumin. Lactose synthetase activity with limiting amounts of the A protein and saturating amounts of α-lactalbumin is defined as LS_A[1] activity (Reaction 3).

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[1] The abbreviations used are: LS_A, lactose synthetase activity; $LS_{(\alpha\text{-LA})}$, lactose synthetase activity with limiting amounts of α-lactalbumin and saturating amounts of the A protein; LS_{end}, endogenous lactose synthetase activity; LacNAc, *N*-acetyllactosamine.

$$\text{UDP-galactose} + \text{glucose} \xrightarrow[\text{limit A}]{\text{excess } \alpha\text{-lactalbumin}} \text{lactose} + \text{UDP} \quad (3)$$

$LS_{(\alpha\text{-LA})}$ activity is with limiting amounts of α-lactalbumin and saturating amounts of the A protein (Reaction 4).

$$\text{UDP-galactose} + \text{glucose} \xrightarrow[\text{limit } \alpha\text{-lactalbumin}]{\text{excess A}} \text{lactose} + \text{UDP} \quad (4)$$

The A protein slowly catalyzes Reaction 1 in the absence of α-lactalbumin (11) and this rate is dependent upon the concentration of glucose as shown in this paper. This rate is referred to as the endogenous lactose synthetase activity (LS_{end}) (Reaction 5).

$$\text{UDP-galactose} + \text{glucose} \xrightarrow[\text{high glucose}]{\text{A protein}} \text{lactose} + \text{UDP} \quad (5)$$

The activity of the A protein in the absence of α-lactalbumin and with *N*-acetylglucosamine instead of glucose as a substrate is referred to as the LacNAc reaction (11) (Reaction 2).

Enzymatic Assays—Recent studies have shown that the assays previously described (4) for LS_A and $LS_{(\alpha\text{-LA})}$ activities required revision (17) and the details of these studies will be reported elsewhere. In particular, it was observed in the LS_A assay that linearity between rate and A protein concentration is observed even though the level of α-lactalbumin is not saturating and that high levels of α-lactalbumin are inhibitory although linearity is still maintained (Fig. 1). The revised conditions for the LS_A assay (spectrophotometric) are as follows: 50 mM glycylglycine or glycine, pH 8.5; 5 mM $MnCl_2$; 0.4 mM UDP-galactose; 25 mM glucose; and 200 μg of α-lactalbumin per ml. The conditions for the LacNAc assay are the same except that the substrate is 25 mM *N*-acetylglucosamine and α-lactalbumin is omitted.

The revised assay conditions (incorporation) for purified bovine α-lactalbumin with purified A protein were: 50 mM glycylglycine or *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid, pH 8.0; 7 mM $MnCl_2$; 0.4 mM UDP-galactose; 100 mM glucose; and

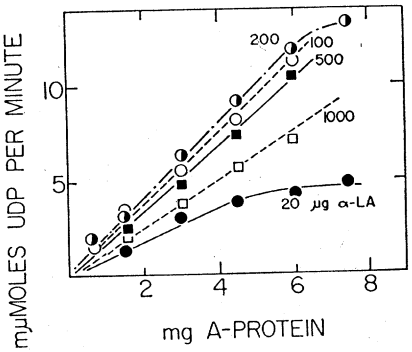

FIG. 1. Spectrophotometric assay for LS_A activity (bovine) at several levels of α-lactalbumin (α-*LA*) (bovine). Assay conditions: A protein off Mannex-P, dissolved in 20 mM Tris-HCl, pH 7.4; 20 mM $MnCl_2$; 20 mM glucose; and 0.25 mM UDP-galactose. ●——●, 20 μg of α-lactalbumin per ml; ○——○, 100 μg of α-lactalbumin per ml; ◑——◑, 200 μg of α-lactalbumin per ml; ■——■, 500 μg of α-lactalbumin per ml; and □——□, 1000 μg of α-lactalbumin per ml.

150 units of A protein measured as LS_A. Activity units are in millimicromoles of UDP formed per min at 25°.

Purification of Bovine A Protein from Skim Milk—The following procedure represents an improvement of the previous procedure (18) in an attempt to obtain larger amounts of the A protein. Six liters of skim milk were made 2 mM in 2-mercaptoethanol, the casein was removed, and a 37 to 50% ammonium sulfate fraction was prepared as previously described (18).

The 37 to 50% $(NH_4)_2SO_4$ precipitate from 12 liters of milk (20 to 30 g of protein in 120 to 160 ml) was dialyzed for 5 hours against 3 to 4 volumes of 10 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. Following dialysis, the conductance of the protein solution was adjusted to that of 20 mM sodium acetate, pH 6.0, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol, by diluting it to a volume of 1 liter with distilled water at 4°. Approximately 150 g of Bio-Rad Cellex-phosphate (hydrogen) were suspended in 1500 ml of 20 mM sodium acetate pH 6.0, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. The protein solution was added batchwise to the Cellex-phosphate suspension and mixed for 30 min at 4°. The gel-protein mixture was transferred to a large column (15 × 30 cm) and allowed to settle. The column was eluted in a stepwise manner as follows: (*a*) 1000 to 1500 ml of 20 mM sodium acetate, pH 6.0, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol; (*b*) 1000 to 1500 ml of 200 mM sodium acetate, pH 6.0, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. About 25% of the total protein added to the Cellex-phosphate was eluted from the column. Some 70 to 75% of the A protein activity was obtained in the 200 mM sodium acetate eluate. Calcium-phosphate gel was added to the solution (2.5 g of gel per g of protein) and the mixture was centrifuged for 20 min at 15,000 × *g* at 0°. The supernatant solution was made 65% saturated with solid $(NH_4)_2SO_4$ (430 g per liter) and the resulting precipitate was resuspended in a minimum volume of 20 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. The protein extract can be frozen at this stage.

The protein solution was dialyzed for 5 hours against 3 to 4 volumes of 10 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. After dialysis the conductance of the protein solution was adjusted to that of 20 mM sodium acetate, pH 5.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol by diluting it with cold distilled water. The protein sample was placed on a carboxymethyl cellulose column (Whatman CM-32, 3 × 17 cm) which had been equilibrated with 20 mM sodium acetate, pH 5.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. Elution was conducted with a linear gradient, with 400 ml of each of 20 mM sodium acetate, pH 5.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol and 200 mM sodium acetate, pH 6.0, also containing $MgCl_2$ and 2-mercaptoethanol. Between 50 and 80% of the activity could be recovered in a broad, diffuse protein peak that began elution at 70 to 80 mM sodium acetate. Fractions containing A activity were pooled and made 65% saturated with solid $(NH_4)_2SO_4$ (430 g per liter). The precipitated protein was centrifuged for 20 min at 15,000 × *g* and resuspended in 20 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol.

Material from the carboxymethyl cellulose column was dialyzed for 5 hours against 3 to 4 volumes of 10 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. The protein solution was then placed on a hydroxylapatite column

(2 × 15 cm), which had been equilibrated with 20 mM Tris, pH 8.5, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. The column was eluted in a stepwise manner with: (*a*) 100 ml of 20 mM Tris, pH 8.5, containing $MgCl_2$ and 2-mercaptoethanol; (*b*) 200 ml of 20 mM Tris, pH 8.5, containing $MgCl_2$ and 2-mercaptoethanol plus 15 mM $(NH_4)_2SO_4$; and (*c*) 250 ml of 20 mM Tris, pH 8.5, containing $MgCl_2$ and 2-mercaptoethanol plus 50 mM $(NH_4)_2SO_4$. Protein off the first hydroxylapatite column was monitored at 220 mμ and 70 to 80% of the total A activity could be recovered in the buffer containing 50 mM $(NH_4)_2SO_4$. This fraction was again precipitated with 65% ammonium sulfate, centrifuged, and resuspended in 2 to 3 ml of 20 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. The material from three to four hydroxylapatite columns was combined, dialyzed for 5 hours against 3 to 4 volumes of 10 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol, and placed on a second hydroxylapatite column (2 × 15 cm), which had been equilibrated with 20 mM Tris, pH 8.5, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol. The column was eluted in a stepwise manner with the same buffers as described for the first hydroxylapatite columns. Approximately 90% of the A activity could be recovered in the buffer containing 50 mM $(NH_4)_2SO_4$. This fraction was precipitated with 65% ammonium sulfate, centrifuged, and resuspended in a minimum volume of 20 mM Tris, pH 7.4, containing 5.0 mM $MgCl_2$ and 2.0 mM 2-mercaptoethanol.

Material from the second hydroxylapatite columns was dialyzed for 5 hours against distilled water, lyophilized, and stored dry at 0°. The final specific activity was between 1 and 3. The over-all yield from the first ammonium sulfate fractionation is about 10% and 8 to 10 mg of purified protein were obtained from 80 liters of skim milk. Chromatography on disc gel electrophoresis in 50 mM sodium phosphate, pH 7.2 (continuous buffer), showed the absence of contaminating proteins. Chromatography on Bio-Gel P-100 did not increase the specific activity and the protein elution peak and activity peak coincided. The molecular weight was estimated to be about 70,000 to 75,000 by chromatography in Sephadex G-100.

RESULTS

Kinetic Studies on LS_A Reaction—With the use of the revised assay, several kinetic experiments were performed to provide further insight into the complexity of the LS_A reaction. The data in Fig. 2 show that the saturation level for UDP-galactose concentration was 0.4 mM but that the fixed level of glucose influences the maximum velocity. When the glucose level was 20 mM, the optimum UDP-galactose concentration was 0.4 mM at either 50 or 200 μg of α-lactalbumin per ml, although the maximum velocity at 200 μg of α-lactalbumin per ml was twice that at 50 μg of α-lactalbumin per ml.

Conversely, similar studies on the effects of UDP-galactose and α-lactalbumin concentration on the optimum glucose concentration were performed. Concentrations between 0.05 and 0.4 mM UDP-galactose at 200 μg of α-lactalbumin per ml did not alter the optimum glucose concentration (20 mM) although maximum activity was obtained with 0.4 mM UDP-galactose. However, the optimum glucose concentration at 50 μg of α-lactalbumin per ml and 200 μg of α-lactalbumin per ml was 50 mM and 20 mM, respectively (Fig. 3). In addition, the maximum activity is higher at 200 μg of α-lactalbumin per ml and 20 mM glucose than at 50 mM glucose and 50 μg of α-lactalbumin per ml. This and other experiments have shown that the concentration of the α-lactalbumin affects the glucose concentration at which LS_A activity is a maximum. The Lineweaver-Burk plots from the data in Fig. 3 gave values of the K_m for glucose at 50 μg of α-lactalbumin per ml and 200 μg of α-lactalbumin per ml of 15.4 mM and 5 mM, respectively. Similar effects of α-lactalbumin concentration on the K_m for glucose are found with other levels of UDP-galactose. These results are: 0.05 mM UDP-galactose, 33 mM (50 μg of α-lactalbumin per ml) and 4 mM (200 μg of α-lactalbumin per ml); 0.1 mM UDP-galactose, 11 mM (50 μg of α-lactalbumin per ml) and 3 mM (200 μg of α-lactalbumin per ml); and 0.2 mM UDP-galactose, 14 mM (50 μg of α-lactalbumin per ml) and 4 mM (200 μg of α-lactalbumin per ml). These plots also showed marked substrate inhibition of enzymatic

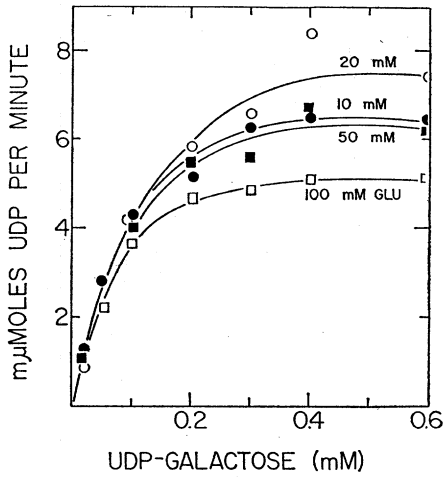

FIG. 2. Optimum UDP-galactose concentration in LS_A assay (spectrophotometric) at a variety of glucose concentrations. Assay conditions: 50 mM glycylglycine, pH 8.5; 5 mM $MnCl_2$; and 200 μg of α-lactalbumin per ml. A protein was from the first hydroxylapatite column chromatography. Glucose concentrations: ●——●, 10 mM; ○——○, 20 mM; ■——■, 50 mM; and □——□, 100 mM.

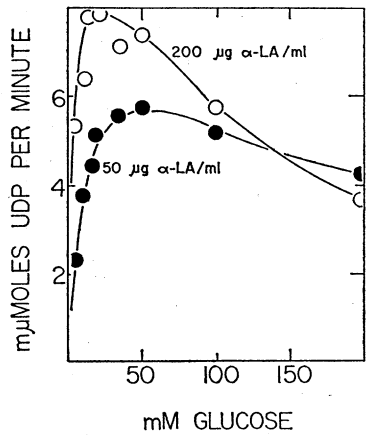

FIG. 3. Glucose optimum in LS_A assay with two α-lactalbumin (*α-LA*) concentrations. Assay conditions: 50 mM glycylglycine, pH 8.5; 5 mM $MnCl_2$; and 0.4 mM UDP-galactose. A protein was from the first hydroxylapatite column. ●——●, 50 μg of α-lactalbumin per ml; ○——○, 200 μg of α-lactalbumin per ml.

activity by glucose. Andrews (19) has shown in human milk that the apparent K_m for glucose is lowered by α-lactalbumin.

Comparison of LS_A and LacNAc Assay Parameters—A comparison of the LacNAc and LS_A assay was made with A protein preparations from the first hydroxylapatite column. The pH optimum for LS_A (25 mM glucose, 0.4 mM UDP-galactose, 5 mM $MnCl_2$, 200 μg of α-lactalbumin per ml in glycylglycine), and LacNAc (25 mM *N*-acetylglucosamine, the others as with LS_A, α-lactalbumin omitted) activities was between pH 8.0 and 9.5

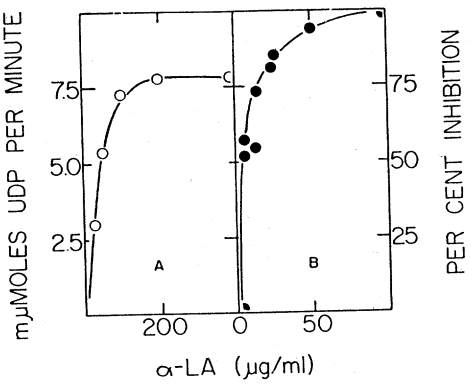

FIG. 4. The role of the α-lactalbumin (α-*LA*) in LS_A and LacNAc assays. *A*, O——O, LS_A activity (rate plotted against α-lactalbumin concentration): 50 mM glycylglycine, pH 8.5; 5 mM $MnCl_2$; 0.4 mM UDP-galactose; 25 mM glucose; and 13 units of A from the first hydroxylapatite column. *B*, ●——●, LacNAc activity (percentage inhibition plotted against α-lactalbumin concentration): 50 mM glycylglycine, pH 8.5; 5 mM $MnCl_2$; 0.4 mM UDP-galactose; 25 mM *N*-acetylglucosamine; and 12 units of A from the first hydroxylapatite column.

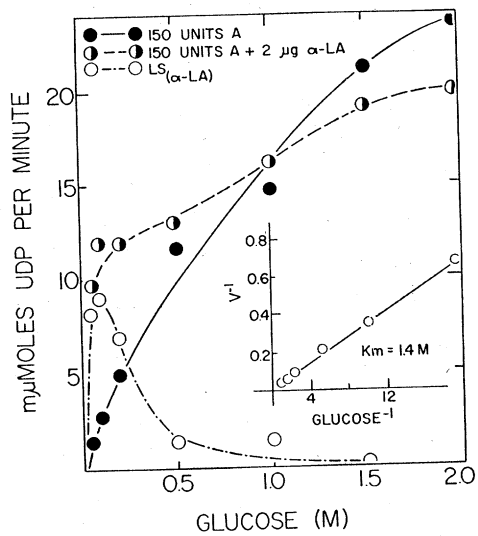

FIG. 5. Effect of glucose concentration on LS_{end} and $LS_{(\alpha\text{-LA})}$ activities with purified bovine proteins. ●——●, LS_{end} activity, for assays with A protein along; ◑——◑, values obtained with A and α-lactalbumin (α-*LA*) present in the assays; and O——O, $LS_{(\alpha\text{-LA})}$ activity obtained by subtracting the values obtained for A alone from the values obtained with both A and α-lactalbumin in the assay. The *inset* shows the Lineweaver-Burk plot for glucose of the LS_{end} assays; the apparent K_m for glucose was 1.4 M. Spectrophotometric assay conditions: 50 mM glycylglycine, pH 8.5; 5 mM $MnCl_2$; 0.4 mM UDP-galactose; 2 μg of α-lactalbumin per ml; and 150 LS_A units per ml of A protein from the first hydroxylapatite column.

although the LacNAc was slightly more active on the acid side. Both activities were optimal with 0.4 mM UDP-galactose, 5 mM $MnCl_2$, and 25 mM glucose or *N*-acetylglucosamine. These results would suggest that the LacNAc and LS_A reaction were catalyzed by the same protein.

α-Lactalbumin inhibits the LacNAc reaction but is required for the LS_A reaction. The data presented in Fig. 4 show that the LS_A reaction is saturated with 200 μg of α-lactalbumin per ml whereas 5 μg of α-lactalbumin per ml inhibit the LacNAc reaction 50%; 100 μg of α-lactalbumin per ml inhibit nearly 100%.

Particles prepared from rabbit gastric mucosa (13) and assayed at a glucose concentration of 25 mM had essentially no lactose synthetase activity in the absence of the bovine α-lactalbumin but had an activity of 8.5 LS_A units per g of tissue in the presence of 200 μg of bovine α-lactalbumin per ml. The results with *N*-acetylglucosamine as substrate were: 16.2 units per g in the absence of α-lactalbumin and 4.7 units per g in the presence of 200 μg of α-lactalbumin per ml. These particles are a rich source of the blood group-active substances and have both α- and β-galactosyl transferases. The observations that these particles catalyze the formation of lactose when glucose and α-lactalbumin are present and that LacNAc synthesis is markedly inhibited by α-lactalbumin give further support to the view that the A protein may be a general galactosyl transferase and that α-lactalbumin modifies the specificity of the galactosyl acceptor (11).

Effect of Glucose on Endogenous Activity of A Protein—Brew *et al.* (11) reported that the A protein had a low endogenous lactose synthetase activity in the absence of added α-lactalbumin. The endogenous activity of the A protein (LS_{end}) must be corrected for in an assay for α-lactalbumin, especially when low levels of α-lactalbumin (1 μg per ml) are being determined. Since α-lactalbumin and glucose concentrations were reciprocally related for maximum activity in the lactose synthetase assay (Fig. 3), the effect of glucose concentration on $LS_{(\alpha\text{-LA})}$ activity was investigated. The data presented in Fig. 5 indicate that maximum $LS_{(\alpha\text{-LA})}$ activity (with purified bovine proteins) occurs with about 0.1 M glucose. Furthermore, Fig. 5 shows that the endogenous activity of the A protein alone is greatly stimulated by high concentrations of glucose. The Lineweaver-Burk plot for the activity of the A protein alone (LS_{end}) is linear between 0.1 and 2.0 M glucose (Fig. 5) and the apparent K_m for glucose is 1.4 M.

It was possible that the apparent activation of the A protein in the LS_{end} assays shown in Fig. 5 was due to nonspecific osmotic effects of the high sugar concentrations. However, 0.2 M mannose, lactose, galactose, or sucrose did not give a rate, nor did their presence influence the rate observed with 0.2 M glucose. Thus, the activation of the LS_{end} activity of the A protein appears to be specific for glucose.

It was possible that the A protein in the presence of the high concentration of glucose was catalyzing the hydrolysis of UDP-galactose to release UDP and galactose. However, appropriate experiments showed that this was not the case and that the product of the A protein in the presence of 100 mM glucose and no α-lactalbumin was lactose.

The possibility existed that the A protein preparations contained traces of α-lactalbumin which in combination with the A protein could account for an endogenous rate. A sample of A protein which had been chromatographed on carboxymethyl

TABLE I

Variable activities of bovine A protein

LS_A assays were with the standard spectrophotometric LS_A assay (13). Assays for LS_{end} and $LS_{(\alpha\text{-LA})}$ activities were with similar conditions except that the glucose concentration was 100 mM. The preparations of A protein were from the first hydroxylapatite column except Preparation E which was from carboxymethyl cellulose. Preparation D was stored frozen at −20°. U = millimicromoles of UDP formed per min.

Sample	LS_A	LS_{end}	$LS_{(\alpha\text{-LA})}$
	U/ml	*U/100 U LS_A*	*U/100 U LS_A/1 μg α-lactalbumin*
A	3000	1.93	14.79
B	4500	3.50	8.24
C	2760	1.64	2.70
D	5700 (Day 0)	1.79 (Day 2)	1.62 (Day 2)
	3760 (Day 7)	3.30 (Day 7)	7.54 (Day 7)
E	1034	45.0	0
F	6000	2.12	3.26

cellulose and hydroxylapatite was further chromatographed on Bio-Gel P-60. The LS_A and $LS_{(end)}$ activities had identical elution profiles from the column and no $LS_{(\alpha\text{-LA})}$ activity was detected at the elution volume of α-lactalbumin.

To investigate further the possibility of contamination of the A protein preparations with α-lactalbumin, the A protein from the Bio-Gel P-60 column was incubated with antiserum to α-lactalbumin. These experiments showed that the antiserum had no significant effect on the $LS_{(end)}$ activity even though it completely removed the $LS_{(\alpha\text{-LA})}$ activity when small amounts of exogenous α-lactalbumin were added to the incubation mixture. These results support the conclusion that the A protein has inherent lactose synthetase activity or that small amounts of α-lactalbumin are so tightly bound to the A protein that no separation occurs upon Bio-Gel P-60 chromatography and no inactivation with antiserum to α-lactalbumin is observed. The latter possibility is highly unlikely since experiments designed to show a stable complex between α-lactalbumin and the A protein have been negative.[2]

During the investigation of the optimum conditions for the lactose synthetase assay considerable variation in the $LS_{(end)}$ activity and in the sensitivity to α-lactalbumin in the $LS_{(\alpha\text{-LA})}$ assay was observed for different preparations of A protein. Some of these results are tabulated and presented in Table I. It is apparent that the various preparations of A protein do not have the same relative activities when the different assays are compared. Furthermore, there is some indication that the relative activity of the A protein under storage conditions in solution may not be constant. Recent studies have shown that the activities of the lyophilized material stored dry at −10° are constant.

DISCUSSION

The LS_A and LacNAc activities have nearly identical optimal assay parameters with respect to pH, $MnCl_2$, UDP-galactose, and substrate (glucose or *N*-acetylglucosamine) concentrations. These findings support the suggestion made by Brew *et al.* (11) that the A protein is involved in the synthesis of both lactose and *N*-acetyllactosamine and that both activities are catalyzed by a single protein. Neither activity was observed when 5 mM $MgCl_2$ was substituted for $MnCl_2$, which is in contrast to the observation of Babad and Hassid (3) who reported that $MgCl_2$ was 25% as effective as $MnCl_2$.

The concentration of α-lactalbumin in bovine milk is about 1 to 3 mg per ml (20) and in bovine mammary tissue it is approximately (minimum) 35 μg per g, wet weight (6). Baldwin and Cheng (21) have determined the glucose levels in the mammary tissue of the bovine and the rat. The concentration of glucose is about 2.4 mM in the lactating bovine and is between 0.4 and 1.0 mM in the lactating rat. In effect, α-lactalbumin lowers the K_m for glucose and hence a high concentration of α-lactalbumin is necessary to ensure maximum synthesis of lactose at the relatively low concentration of glucose found in tissue. It is conceivable that the amount of lactose found in a given milk is influenced by the ability of the α-lactalbumin to lower the K_m for glucose so that maximum synthesis may proceed. At present, it is not clear what regulates the lactose content of milk (22).

Andrews (19) has observed in a crude preparation of the A protein from human milk that the K_m for glucose was lowered from 60 mM in the absence to 3 mM in the presence of α-lactalbumin.

In the bovine system, the K_m for glucose is lowered from 1400 mM in the absence to 5 mM in the presence of α-lactalbumin. Brew *et al.* (11) have previously reported that a partially purified bovine A protein had a very low endogenous lactose synthetase activity in the absence of α-lactalbumin. The present study with the purified bovine A protein shows that glucose is a very poor substrate and that mannose, galactose, and sucrose were not substrates. It is evident that the glucose concentration in the $LS_{(\alpha\text{-LA})}$ assay must be kept low in order to minimize the endogenous activity of the A protein.

Different preparations of the A protein have variable activities at the same stage of purification (Table I). One possible explanation is that the A protein is not a homogeneous protein but may consist of a number of galactosyl transferases which have slightly different substrate specificities and kinetic properties. Indeed, galactosyl transferases which have a broad acceptor specificity exist in particulate fractions of tissues and it is uncertain whether this activity represents one or more enzymes (12, 23). The A protein is also present in the particulate fraction of other tissues (24) and will synthesize lactose in the presence of α-lactalbumin.

Another possibility is that the A protein may exist in multiple, easily interconvertible forms. The latter proposal may be more consistent with the changing activity which occurs with a single A preparation upon storage. Palmiter (25) has shown that the A protein (LacNAc synthetase activity) from mouse mammary glands has three different molecular weights, and it would be of interest to ascertain whether they all had lactose synthetase activity. The resolution of these possibilities awaits more complete purification and characterization of the A protein from a variety of sources.

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